## SEQUENCE LISTING

```
<110> Odyssey Thera, Inc.
      Michnick, Stephen W.
      Remy, Ingrid
      Lamerdin, Jane
      You, Helen
      Westwick, John
      MacDonald, Marnie L.
<120> PROTEIN FRAGMENT COMPLEMENTATION ASSAYS FOR HIGH-THROUGHPUT AND
      HIGH-CONTENT SCREENING
<130> ODDY006
<150> US60/445,225
<151> 2003-02-06
<150> US10/353,090
<151> 2003-01-29
<150> US10/154,758
<151> 2002-05-24
<150> US09/499,464
<151> 2000-02-07
<150> US09/017,412
<151> 1998-02-02
<160> 31
<170> PatentIn version 3.2
<210> 1
<211> 10
<212> PRT
<213> Artificial
<220>
<223> synthetic construct, a flexible linker
<400> 1
Gly Gly Gly Ser Gly Gly Gly Ser
<210> 2
<211> 483
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; RLuc(F1) with stop codon added at end
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<220> <221> CDS <222> (1)(4) <223> RLuc(F1)	<ol> <li>corresponds</li> </ol>	to a.a. r	residues 1-160	of wild-type	∋ R.
		Pro Glu G	caa cgc aaa cgo Gln Arg Lys Arg LO		
	rp Trp Ala Arg		caa atg aac gtg Gln Met Asn Va		
			cac gcc gag aad His Ala Glu Ası 45		
		_	tac ctg tgg agg Tyr Leu Trp Arg 60		-
			atc atc cct ga Ile Ile Pro Asp 75		
		Gly Asn G	ggc tca tat cgo Gly Ser Tyr Arg 90		
His Tyr Lys Ty	_	-	gag ctg ctg aad Glu Leu Leu Asi		-
			ggg gct tgt ctg Gly Ala Cys Len 12	ı Ala Phe Hi	
		Lys Ile L	aag gcc atc gto Lys Ala Ile Vai 140		
			gac gag tgg cc Asp Glu Trp Pro 155		u
taa			•		483

<211> 160

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 3

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser 20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  $35 \hspace{1cm} 40 \hspace{1cm} 45$ 

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val 50 55 60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly 65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp 85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys
100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His 115 120 125

Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu 130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu 145 150 155 160

<210 <211 <212 <213	L> 4 2> I	1 180 DNA Artif	ficia	al												
<220	)>															
<223	3> \$			c cor "ato						ith s	stop	code	on ad	dded	at e	end and
	L> ( 2>	CDS (1)		)) cori	cespo	onds	to a	a.a.	res:	idues	s 1-1	L60 d	of wi	ild-1	tvpe	R.
		Lucii													-11	
	tcc			tac Tyr 5												48
				gct Ala												96
				gat Asp												144
				gct Ala												192
				gtg Val												240
				aag Lys 85												288
				acc Thr												336
				ggc Gly												384
				caa Gln												432
				atc Ile											taa	480

<210> 5 <211> 159 <212> PRT <213> Artificial <220> <223> Synthetic Construct <400> 5 Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr Gly 10 Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser Phe 25 20 Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile Phe 35 40 Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val Pro 55 His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly Met 65 70 75 Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp His 90 95 85 Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys Lys 100 105 Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His Tyr

Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu Ser

Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu

135

150

145

155

140

<210> <211> <212> <213>	6 459 DNA Arti	ficia	al												
<220> <223>	_									-					added agment
<220><221><222><222><223>		(F2)	cor	respo	onds	to a	a.a.	resi	idues	s 161	1-31	l of	wild	d-typ∘	e R.
<400> atg ga Met Gl															48
ctt ga Leu Gl															96
cgg aa Arg Ly															144
gag aa Glu Ly 50															192
cct ct Pro Le 65															240
tac aa Tyr As				Arg		Ser	Asp	Asp		Pro	Lys	Met			288
gag tc Glu Se															336
aag tt Lys Ph															384
cag ga Gln Gl 13	u Asp	_		_	_	_		_			_	-			432
gag cg Glu Ar 145															459

<211> 152

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 7

Met Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val 1 5 10 15

Leu Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met 20 25 30

Arg Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys 35 40 45

Glu Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile 50 55 60

Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn 65 70 75 80

Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile 85 90 95

Glu Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys
100 105 110

Lys Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser 115 120 125

Gln Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val 130 135 140

Glu Arg Val Leu Lys Asn Glu Gln 145 150

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<210> 8
<211> 456
<212> DNA
<213>
      Artificial
<220>
<223> synthetic construct; RLuc(F2) with a stop codon added at the end
       of the fragment
<220>
<221>
      CDS
<222>
      (1)..(456)
      RLuc(F2) corresponds to a.a. residues 161-311 of wild-type R.
<223>
       Luciferase
<400> 8
gag gat atc gcc ctg atc aag agc gaa gag ggc gag aaa atg gtg ctt
                                                                       48
Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu
gag aat aac ttc ttc gtc gag acc atg ctc cca agc aag atc atg cgg
                                                                       96
Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg
            20
                                25
aaa ctg gag cct gag gag ttc gct gcc tac ctg gag cca ttc aag gag
                                                                      144
Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu
        35
                            40
aag ggc gag gtt aga cgg cct acc ctc tcc tgg cct cgc gag atc cct
                                                                      192
Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro
    50
ctc gtt aag gga ggc aag ccc gac gtc gtc cag att gtc cgc aac tac
                                                                      240
Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr
65
                                                             80
aac gcc tac ctt cgg gcc agc gac gat ctg cct aag atg ttc atc gag
                                                                      288
Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu
                85
tcc gac cct ggg ttc ttt tcc aac gct att gtc gag gga gct aag aag
                                                                      336
Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys
            100
                                105
ttc cct aac acc gag ttc gtg aag gtg aag ggc ctc cac ttc agc cag
                                                                      384
Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln
                            120
        115
gag gac gct cca gat gaa atg ggt aag tac atc aag agc ttc gtg gag
                                                                      432
Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu
                        135
    130
                                             140
                                                                      456
cgc gtg ctg aag aac gag cag taa
Arg Val Leu Lys Asn Glu Gln
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145 150

<210> 9

<211> 151

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 9

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu
1 5 10 15

Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg 20 25 30

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu 35 40 45

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro 50 55 60

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr 65 70 75 80

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu 85 90 95

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys 100 105 110

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln 115 120 125

Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu 130 135 140

Arg Val Leu Lys Asn Glu Gln 145 150

```
<210> 10
<211>
      483
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; RLuc(F1) with a C124A mutation and stop
       codon at end
<220>
<221>
      CDS
<222>
      (1)..(483)
<400> 10
atg get tee aag gtg tae gae eee gag caa ege aaa ege atg ate aet
                                                                       48
Met Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr
                                    10
ggg cct cag tgg tgg gct cgc tgc aag caa atg aac gtg ctg gac tcc
                                                                       96
Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser
            20
ttc atc aac tac tat gat tcc gag aag cac gcc gag aac gcc gtg att
                                                                      144
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile
        35
ttt ctg cat ggt aac gct gcc tcc agc tac ctg tgg agg cac gtc gtg
                                                                      192
Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val
cct cac atc gag ccc gtg gct aga tgc atc atc cct gat ctg atc gga
                                                                      240
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly
atg ggt aag too ggc aag ago ggg aat ggc toa tat ogo oto otg gat
                                                                      288
Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp
                                    90
cac tac aag tac ctc acc gct tgg ttc gag ctg ctg aac ctt cca aag
                                                                      336
His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys
            100
                                105
                                                     110
aaa atc atc ttt gtg ggc cac gac tgg ggg gct gct ctg qcc ttt cac
                                                                      384
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His
        115
                            120
tac tcc tac gag cac caa gac aag atc aag gcc atc gtc cat gct gag
                                                                      432
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu
    130
                        135
agt gtc gtg gac gtg atc gag tcc tgg gac gag tgg cct gac atc gag
                                                                      480
Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu
145
                    150
                                         155
                                                             160
taa
                                                                      483
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<211> 160

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 11

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser 20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  $35 \hspace{1cm} 40 \hspace{1cm} 45$ 

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val 50 55 60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly 65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp 85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys 100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His 115 120 125

Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu 130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu 145 150 155 160

```
<210> 12
<211> 480
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; RLuc(F1) with C124A mutation, initiating
       "atg" removed, and stop codon at end
<220>
<221> CDS
<222>
     (1)..(480)
<400> 12
gct tcc aag gtg tac gac ccc gag caa cgc aaa cgc atg atc act ggg
                                                                       48
Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr Gly
cct cag tgg tgg gct cgc tgc aag caa atg aac gtg ctg gac tcc ttc
                                                                       96
Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser Phe
            20
                                25
                                                    30
atc aac tac tat gat tcc gag aag cac gcc gag aac gcc gtg att ttt
                                                                      144
Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile Phe
        35
                            40
ctg cat ggt aac gct gcc tcc agc tac ctg tgg agg cac gtc gtg cct
                                                                      192
Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val Pro
                        55
cac atc gag ccc gtg gct aga tgc atc cct gat ctg atc gga atg
                                                                      240
His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly Met
ggt aag tcc ggc aag agc ggg aat ggc tca tat cgc ctc ctg gat cac
                                                                      288
Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp His
tac aag tac ctc acc gct tgg ttc gag ctg ctg aac ctt cca aag aaa
                                                                      336
Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys Lys
            100
                                105
atc atc ttt gtg ggc cac gac tgg ggg gct gct ctg gcc ttt cac tac
                                                                      384
Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His Tyr
        115
                            120
                                                125
tcc tac gag cac caa gac aag atc aag gcc atc gtc cat gct gag agt
                                                                      432
Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu Ser
    130
gtc gtg gac gtg atc gag tcc tgg gac gag tgg cct gac atc gag taa
                                                                      480
Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu
145
                    150
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<211> 159

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 13

Ala Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr Gly
1 5 10 15

Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser Phe 20 25 30

Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile Phe 35 40 45

Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val Pro 50 55 60

His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly Met 65 70 75 80

Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp His 85 90 95

Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys Lys
100 105 110

Ile Ile Phe Val Gly His Asp Trp Gly Ala Ala Leu Ala Phe His Tyr 115 120 125

Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu Ser 130 135 140

Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu 145 150 155

```
<210> 14
<211> 477
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; YFP(F1) with added stop codon at end
<220>
<221> CDS
<222>
      (1)...(477)
<223> YFP(F1) corresponds to a.a. 1-158 of the full length EYFP
<400> 14
atg gtg agc aag ggc gag gtg ctg ttc acc ggg gtg gtg ccc atc ctg
                                                                       48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc
                                                                       96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20
gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc
                                                                      144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35
                            40
tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc
                                                                      192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
ttc ggc tac ggc ctg cag tgc ttc gcc cgc tac ccc gac cac atg aag
                                                                      240
Phe Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag
                                                                      288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                                    90
cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag
                                                                      336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100
                                105
                                                     110
gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc
                                                                      384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115
                            120
atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac
                                                                      432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130
aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag taa
                                                                      477
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
145
                    150
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<210> 15 <211> 158

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 15

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu 5

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly 25

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile 40

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr 50 55

Phe Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys 70 75

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu 85 90

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu 100 105

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly 115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr 130 135

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln 145 150 155

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<210> 16
<211>
      474
<212>
      DNA
<213> Artificial
<220>
<223> synthetic construct; YFP(F1) with stop codon added at end and
       initial "atg" (or Met) removed
<220>
<221> CDS
<222>
      (1)...(474)
<223> YFP(F1) corresponds to a.a. 1-158 of the full length EYFP
<400> 16
gtg agc aag ggc gag gtg ttc acc ggg gtg gtg ccc atc ctg gtc
                                                                       48
Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
                                    10
gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag
                                                                       96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc
                                                                      144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35
                            40
                                                45
acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ttc
                                                                      192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50
                        55
ggc tac ggc ctg cag tgc ttc gcc cgc tac ccc gac cac atg aag cag
                                                                      240
Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln
65
cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc
                                                                      288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85
                                    90
acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg
                                                                      336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100
                                105
aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc
                                                                      384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115
                            120
gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac
                                                                      432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130
                        135
tac aac agc cac aac gtc tat atc atg gcc gac aag cag taa
                                                                      474
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
145
                    150
                                        155
```

<211> 157

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 17

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val 1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu 20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys 35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe 50 55 60

Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val 100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn 130 \$135\$

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln 145 150 155

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<210> 18
<211> 249
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; YFP(F2) with added "atg" (or Met) at
       position 1 and stop codon at end
<220>
<221> CDS
<222> (1)..(249)
<223> YFP(F2) corresponds to a.a. 159-239 of the full length EYFP
<400> 18
atg aag aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag
                                                                      48
Met Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu
gac ggc agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc
                                                                      96
Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile
            20
                                25
ggc gac ggc ccc gtg ctg ccc gac aac cac tac ctg agc tac cag
                                                                     144
Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln
        35
                            40
                                                45
tcc gcc ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg
                                                                     192
Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu
    50
                        55
ctg gag ttc gtg acc gcc gcg atc act ctc ggc atg gac gag ctg
                                                                     240
Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu
65
                    70
                                        75
                                                            80
tac aag taa
                                                                     249
Tyr Lys
<210> 19
<211> 82
<212> PRT
<213> Artificial
<220>
<223> Synthetic Construct
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## <400> 19

Met Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu

1 10 15

Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile
20 25 30

Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln 35 40 45

Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu 50 60

Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu 65 70 75 80

Tyr Lys

<210> 20

<211> 246

<212> DNA

<213> Artificial

<220>

<223> synthetic construct; YFP(F2) with added stop codon at end

<220>

<221> CDS

<222> (1)..(246)

<223> YFP(F2) corresponds to a.a. 159-239 of the full length EYFP

<400> 20

aag aac ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp
1 5 10 15

ggc agc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly

20 25 30

gac ggc ccc gtg ctg ctc gac aac cac tac ctg agc tac cag tcc

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser

35

40

45

gcc ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
50 55 60

		gtg Val														240
aag Lys	taa															246
<210 <211 <212 <213	L> :	21 81 PRT Artii	ficia	al												
<220 <223		Syntl	netio	c Cor	nstrı	ıct										
<400	)> :	21														
Lys 1	Asn	Gly	Ile	Lys 5	Val	Asn	Phe	Lys	Ile 10	Arg	His	Asn	Ile	Glu 15	Asp	
Gly	Ser	Val	Gln 20	Leu	Ala	Asp	His	Tyr 25	Gln	Gln	Asn	Thr	Pro 30	Ile	Gly	
Asp	Gly	Pro 35	Val	Leu	Leu	Pro	Asp 40	Asn	His	Tyr	Leu	Ser 45	Tyr	Gln	Ser	
Ala	Leu 50	Ser	Lys	Asp	Pro	Asn 55	Glu	Lys	Arg	Asp	His 60	Met	Val	Leu	Leu	
Glu 65	Phe	Val	Thr	Ala	Ala 70	Gly	Ile	Thr	Leu	Gly 75	Met	Asp	Glu	Leu	Tyr 80	
Lys																

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<210> 22
<211> 477
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; IFP(F1) with stop codon added at end
<220>
<221> CDS
<222> (1)..(477)
<223> IFP(F1) corresponds to a F46L mutated form of SEYFP(F1)
<400> 22
atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg
                                                                       48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
                                    10
gtc gag ctg gac ggc gta aac ggc cac aag ttc agc gtg tcc ggc
                                                                       96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20
gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttg atc
                                                                      144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile
        35
tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc
                                                                      192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
ctc ggc tac ggc ctg cag tgc ttc gcc cgc tac ccc gac cac atg aag
                                                                      240
Leu Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
65
                    70
cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag
                                                                      288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                                    90
cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag
                                                                      336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100
                                105
gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc
                                                                      384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
                            120
                                                                      432
atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130
                        135
aac tac aac agc cac aac gtc tat atc acg gcc gac aag cag taa
                                                                     477
Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
145
                    150
                                        155
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<211> 158

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 23

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu  $1 \hspace{1.5cm} 5 \hspace{1.5cm} 10 \hspace{1.5cm} 15$ 

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly 20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile  $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45$ 

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr 50 55 60

Leu Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu 85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu 100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly 115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr 130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln 145 150 155

```
<210> 24
<211> 474
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; IFP(F1) with initiating "atg" (or Met)
       removed and stop codon added at end
<220>
<221> CDS
<222>
      (1)..(474)
<223>
      IFP(F1) corresponds to a F46L mutated form of SEYFP(F1)
<400> 24
gtg agc aag ggc gag gtg ttc acc ggg gtg gtg ccc atc ctg gtc
                                                                       48
Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag
                                                                       96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20
ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttg atc tgc
                                                                      144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys
                            40
acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ctc
                                                                      192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
                                            60
ggc tac ggc ctg cag tgc ttc gcc cgc tac ccc gac cac atg aag cag
                                                                      240
Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln
cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc
                                                                      288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85
                                    90
acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg
                                                                      336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100
aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc
                                                                      384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115
                            120
gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac
                                                                      432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130
tac aac agc cac aac gtc tat atc acg gcc gac aag cag taa
                                                                      474
Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln
145
                    150
                                        155
```

<211> 157

<212> PRT

<213> Artificial

<220>

<223> Synthetic Construct

<400> 25

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val 1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu 20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys 35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu 50 55 60

Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn 130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln 145 150 155

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<210> 26
<211> 249
<212> DNA
<213> Artificial
<220>
<223> synthetic construct; IFP(F2) with added "atg" (or Met) at
       position 1 and a stop codon at the end
<220>
<221> CDS
<222> (1)..(249)
<223> IFP(F2) corresponds to a V163A, S175G mutated form of YFP(F2)
<400> 26
atg aag aac ggc atc aag gcg aac ttc aag atc cgc cac aac atc gag
                                                                      48
Met Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu
gac ggc ggc gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc
                                                                      96
Asp Gly Gly Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile
            20
ggc gac ggc ccc gtg ctg ccc gac aac cac tac ctg agc tac cag
                                                                     144
Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln
        35
                            40
tcc gcc ctg agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg
                                                                     192
Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu
    50
                        55
ctg gag ttc gtg acc gcc gcg gtg atc act ctc ggc atg gac gag ctg
                                                                     240
Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu
65
                    70
tac aag taa
                                                                     249
Tyr Lys
<210> 27
<211> 82
<212> PRT
<213> Artificial
<220>
<223>
      Synthetic Construct
<400> 27
Met Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu
                                    10
```

Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln 35 40 45
Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu 50 55 60
Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu 65 70 75 80
Tyr Lys
<210> 28 <211> 246 <212> DNA <213> Artificial
<220> <223> synthetic construct; IFP(F2) with an added stop codon at the end
<220> <221> CDS <222> (1)(246)
<223> IFP(F2) corresponds to a V163A, S175G mutated form of YFP(F2)
<223> IFP(F2) corresponds to a V163A, S175G mutated form of YFP(F2)  <400> 28  aag aac ggc atc aag gcg aac ttc aag atc cgc cac aac atc gag gac  Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
<pre>&lt;223&gt; IFP(F2) corresponds to a V163A, S175G mutated form of YFP(F2)  &lt;400&gt; 28 aag aac ggc atc aag gcg aac ttc aag atc cgc cac aac atc gag gac 48 Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp 1</pre>
<pre>&lt;223&gt; IFP(F2) corresponds to a V163A, S175G mutated form of YFP(F2)  &lt;400&gt; 28 aag aac ggc atc aag gcg aac ttc aag atc cgc cac aac atc gag gac 48 Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp 1</pre>
<pre>&lt;223&gt; IFP(F2) corresponds to a V163A, S175G mutated form of YFP(F2)  &lt;400&gt; 28 aag aac ggc atc aag gcg aac ttc aag atc cgc cac aac atc gag gac 48 Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp 1</pre>

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<210> 29
<211> 81
<212> PRT
<213> Artificial
<220>
<223> Synthetic Construct
<400> 29
Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp
                                  10
Gly Gly Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
           20
                               25
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser
       35
                           40
                                              45
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr
                                      75
Lys
<210> 30
<211> 15
<212> PRT
<213> Artificial
<220>
<223> synthetic construct, a flexible linker
<400> 30
Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
```

5

```
<210> 31
<211> 5
<212> PRT
<213> Artificial
<220>
<223> synthetic construct, "5-mer" building block for flexible linkers
<400> 31

Gly Gly Gly Gly Ser
1 5
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Group Art Unit: to be assigned Examiner: to be assigned

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re of the Application of

Stephen Michnick et al

Serial Number: unassigned •

Filed: February 5, 2004

Filed: February 5, 2004

FOR: PROTEIN FRAGMENT

COMPLEMENTATION ASSAYS

FOR HIGH THROUGHPUT AND

HIGH CONTENT SCREENING

HIGH CONTENT SCREENING

## STATEMENT UNDER 37 C.F.R. 1.821(f)

Hon. Commissioner of Patents and Trademarks Alexandria, VA 22313

Dear Sir:

In regards to the above-captioned patent application and sequence listing (both paper copy and computer readable format) filed simultaneously herewith, Applicants' agent attests that the information recorded in the computer readable form is identical to the written sequence listing as required under 37 C.F.R. 1.821(f).

Respectfully submitted,

Reg. No. 29,765

Date: February 5, 2004 2001 Jefferson Davis Highway--Suite 301 Arlington, VA 22202 (703) 418-2777 Oddy 006a